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Antibacterial Activity of the Root Extracts of Spear Grass (*Imperata cylindrica*) in Makurdi Metropolis

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Abstract

The phytochemicals present in *Imperata cylindrica* were investigated as well as their antibacterial activity on *Staphylococcus aureus* and *Escherichia coli* using agar well diffusion method. Both ethanolic and aqueous extractions were carried out. The ethanolic extracts of *Imperata cylindrica* exhibited the highest zones of inhibition against all the bacterial isolates with 18.3 ± 1.53 at 200 Mg/mL against *Staphylococcus aureus* and 16.0 ± 1.00 at 200 mg/mL against *E. coli.* All the extracts followed the trend of "the higher the concentration the greater the zones of inhibition". The minimum inhibitory concentration was observed to be 25% on both *E. coli* and *Staphylococcus aureus* in the ethanolic extract and 50% against *E. coli* and 25% for *Staphylococcus aureus* in the aqueous extract. The result from this study revealed that *Imperata cylindrica* roots contain some bioactive compounds which may be used as antibacterial agents.

Key words: Antibacterial, Activity, Root, Extracts, Spear grass, Phytochemicals, Inhibitory, Concentration

Introduction

Imperata cylindrica is one of the most dominant weeds in Agricultural and non-Agricultural fields in West Africa. According to [1], it is ranked as the world's seventh worst weed. Some of its vernacular names include spear grass, imperata, cogon grass, alang-alang, lalang, blade grass, blade grass, cotton wool grass, ihila, thatch grass, silver spike, among others [2]. Due to its strong competition with crops, *Imperata cylindrica* is considered a harmful perennial grass responsible for serious crop yield losses depending on the type of crop, cultural practices and environmental conditions (Solepardi, [3]. In Nigeria, [4] reported that *Imperata cylindrica* has the potential to invade 260 million hectares of land especially in the moist Savanna and humid forest zones. *Imperata cylindrica* is widely distributed throughout the world. [2] reported that it is widely found in the tropical and subtropical zones of Africa, the Indian subcontinent, South-East Asia and Australia. It occurs to a lesser extent in North, Central and South America and warm temperate areas of New Zealand and Japan. In Africa, it occurs in almost all countries including Northern Africa and South Africa.

Imperata cylindrica has so many uses. The tender new growth can be used as short term supplemental or emergency pasture [5]. The fibrous roots contain starch and sugar and are pleasant to chew [5]. In Lesotho, the rhizomes are eaten raw by herdsmen and in Kenya; Kipsigi children chew them for their sweet flavour. Although attempts have been made to ferment the rhizomes into a beer and to extract sugar and alcohol from them but without commercial success, the aerial parts and the dried rhizomes can be powdered and used as sweetener. It is used for mulching, for instance in coffee and banana plantations and the fast growing rhizomes makes it suitable for erosion control and stabilizing slope *Imperata cylindrica* has so many uses such as edible uses which include the tender new growth which can be used as short term supplemental or emergency pasture, and is used to cure fever, toothache and other disorders [5]. In Nigeria, the Tiv and Igala people bind the grass in rolls and place the rolls on the roof as thatch [2]. Apart from edible uses, the medicinal uses include antibacterial, antihelmintic, astringent [5].

Parasitoses have been a concern to the medical field for centuries and the helminthes are still the cause of considerable problems for human beings and animals. Anthelmintic are drugs which may kill (Vermicide) or expel (Vermifuge) infesting helminthes [6].

It is an important drug of Tripanchmool and used in urinary calculi, retention of urine, diabetes, cardiac disorders, gout, common cough and cold, anemia. It is effective in conditions like arthritis, diarrhea, dysentery, gonorrhea, cancer, diuretic, emollient, febrifuye, restorative, sialagogue, styptic and tonic [7]. *Imperata cylindrica* is a popular herbal medicine in China with the name "Baimaogen", and has been used as a diuretic and

anti-inflammatory agent in traditional Chinese medicine [7].
Imperata cylindrica has a range of traditional medicinal uses throughout tropical Africa. A decoction (extract) of the rhizome is widely drunk as a diuretic, to purify blood and to treat dysentery, colic, hypertension and venereal diseases. Rhizome maceration is taken to treat cough and the pulped-up plant with Shea butter is used as an embrocation for coughs [5].
A decoction of the leaves is drunk to treat furuncles (pus-filled sore) and Candidiasis. In Senegal, Fula people drink a rhizome decoction to treat Schistosomiasis. In Congo, ash of burnt roots is rubbed into scarification to treat chest pain with high fever. Roots are eaten as a galactagogue (a substance that induces lactation). Roasted and powdered roots extracts is taken to treat jaundice and digestive problem. In Southern Africa, a crushed root infusion is taken to treat hiccups and indigestion. In Madagascar, a decoction of the dried plants is taken as mouthwash for sore throat, neuralgia (an acute, severe pain that radiates along a nerve) and intestinal worms. In Namibia, the ground stems are used as a cosmetic [2].
Previous phytochemical studies on the isolation of coumarin, flavones, chromones and other phenolics exhibited diverse pharmacological activities such as cytotoxic, neuro-protective and vasodilative activities [9]. The major chemical constituent in *Imperata cylindrica* includes carbohydrates, glycosides, flavonoids, triterpenoids [5].
Although *Imperata cylindrica* is widely considered a serious weed, it can be useful for various purposes.
There are a number of studies about *Imperata cylindrica* with most of them emphasizing on the Agricultural value and how it can be controlled because of its devastating effect on crops. The aerial part of the grass is given more research attention even when it comes to the medical aspect of it. A broad range of medicinal uses has been reported but most claims still need to be validated. Little has been done in regards to the roots of the grass.
This paucity of knowledge of the phytochemical constituents and the antimicrobial activities of the roots of *Imperata cylindrica* has resulted to its underutilization. The result of this study will initiate the exploitation of the therapeutic potential in the roots.

Materials and Methods

Study Area

The study was carried out in Makurdi, the capital city of Benue State, Nigeria. The city is located in the middle belt region along the Benue River and holds the base for the Nigeria's Air Force's MIG 21 and SEPECAT Jaguar air craft squadrons. In 2015, Makurdi had an estimated population of 342,500 [10]. Makurdi is located on lat. 7°43 and 8° 32'E and long. 7.7°30' and 5.6^0 N and 11° E, the major ethnic groups in Makurdi are Tiv, Idoma, Igede and Etulo. The city is also housing Benue State University and the Federal University of Agriculture. Benue state is an Agricultural area specializing in cash and subsistence crops [10].

Sample collection/extraction

The fresh roots of *I. cylindrica* were collected around Makurdi metropolis. They were washed with clean water to remove sand and properly identified and authenticated in the Botany Department University of Agriculture, Makurdi, Benue State.
The extraction of the crude extract of *I. cylindrica* was carried out following the protocol earlier outlined by [11]. Briefly, air dried roots of *I. cylindrica* were grinded into fine powder and then soaked into ethanol and distilled water. In this method, 100g of the grinded plant material was soaked into 700 mL of each of the two solvent (water and ethanol) and allowed to stay for three days. The extract was filtered through a sieve to remove the debris and the filtrate was then filtered through a filter paper. The facial filtrate was evaporated in a water bath at 40° C to get the crude extract. The aqueous and ethanolic extracts were stored at room temperature until required. These extracts were used for the phytochemical screening and the antibacterial analysis. The bacterial isolates used were obtained from the Microbiology Laboratory Department of Microbiology University of Agriculture Makurdi, Benue State.

Phytochemical Analysis

The aqueous and ethanolic extracts were used to perform the phytochemical screening using standard methods as described by [12]. Exactly 1 ml of the extracts was added to little amount of dilute hydrochloric acid in a test tube and Mayer's reagents were added to the solution, the formation of a white precipitate indicated the presence of alkaloids. Precisely 3 mL of the extract were mixed with a little quantity of a throne reagent in a test tube, drops of concentrated sulphoric acid were added and made into a paste and heated gently over a water bath, a dark green colouration indicated the presence of reducing sugar.

Exactly 2 ml of the extract was dissolved in 2 mL of glacial acetic acid containing a drop of $Fecl_2$ solution was added followed by 2 mL of sulphoric acid. A brown ring formed at the interphase indicated the presence of glycosides. Approximately 2 ml of sodium hydroxide solution were added to 2 mLof the extract. The appearance of a yellow colour indicated the presence of flavonoids. Equal volumes of the extracts and ferric chloride were mixed. A deep bluish green solution indicated the presence of phenol. Approximately 3 mL of extracts were added to about ten drops of ferric chloride.

A brownish green or blue black colour indicated the presences of tannin. Equal amount of the extract and distilled water was shaken vigarously. Foamy lather (Froth) formation indicated the presence of saponins.

To 2 mL of the extract, sodium hydroxide was added. A blue green or red colour indicated the presence of quinones. Few drop of Ninhydric reagent were added to 2 mL of the extract. Appearance of a purple colour indicated the presence of amino acid. The extract was dissolved in 90% ethanol and drops of ferric chloride were added. A green colour formed indicated the presence of volatile oil.

Exactly 5 mL of each extract was mixed in 2 mL of chloroform and 3 mL of concentrated Sulphric acid was carefully added to form a layer. A reddish brown colour indicated the presence of terpenoids. Cold slurred agar slants cultures of already identified one Gram positive organisms (*Staphylococcus aureus*) and one Gram negative organisms (*Escherichia coli*) were used in this study.

Viability test of each isolate was carried out by resuscitating the organisms in buffered peptone broth and thereafter sub-culturing onto nutrient agar medium and incubating at 37° C for 24 hours. The probable identity of the clinically sourced isolates was further confirmed by exposing the cultures to an array of standard biochemical tests which included coagulase, catalase, indole, methyl red and citrate utilization tests as described by [13] and [14]. The result of the biochemical reactions elicited by the test isolates as described by the test isolates were compared to standard identification keys as described [13].

One gram (1g) each of the aqueous and ethanolic extracts was added to 5 mL of distilled water and ethanol respectively to give a concentration of 200 mg/mL. Other concentration of 100 mg/mL 50mg /mL, 25mg/mL and 12.5 mg/mL were prepared by double dilution method as described by [15].

Susceptibility testing was carried out using agar diffusion method. In this method, the inoculums were prepared by inoculating the test organisms in nutrient broth and they were incubated for 24 hours at 37° C. The cultures were diluted to 0.5 McFarland Standard Turbidity after incubation. Exactly 0.5cm each of the cultured organisms was pipetted onto the culture plates after which prepared Mueller Hinton agar was pour plated on the plates and allowed to solidify.

After the culture plates have gelled, wells were bored on the surface of the agar plates using 4mm cork-borer. About 0.2 mL of the different concentration of each extract was transferred into the well using Pasteur pipette. These wells were sufficiently spaced to prevent the resulting zones of inhibition from overlapping. The plates were incubated at 37° C for 24 hours. The experiment was performed in triplicate and the resulting zones of inhibition recorded as mean and standard deviation.

The Minimum Inhibitory Concentration (MIC) of the potent extracts was determined according to the macro broth dilution technique (Baron and fine gold, 1990). Standardized suspension of the test organisms were inoculated into a series of sterile test tubes of nutrient broth containing two fold dilutions the root extract and incubated at 37° C for 24 hours. The MICs were recorded as the least concentration that inhibited the growth of the least organisms.

The Minimum Bactericidal Concentration (MBC) of the respective root extracts was determine by procedure described by [16]. Aliquot (1ml) was taken from the MIC tubes with no visible growth and subculture on freshly prepared nutrient agar plates and later incubated at 37° C for 24 hours. The MBC was taken as the concentration of the extract that did not show any growth on a new set of agar plates.

Data analyzed for mean and standard deviation difference in parameter was tested for statistical difference at $P \leq 0.05$ Using student t-test. All Analysis was done using statistical package Service solution (SPSS).

Results and Discussion

The result of the analysis is shown in Tables 1, 2, 3 and 4. Table 1 shows the phytochemical constituents of *Imperata cylindrica* roots extracts using ethanol and distilled water and these include; alkaloids, reducing sugars, cardiac glycosides, flavonoids, tannins terpenoids and saponins.

Tables 2 and 3 show the result of the antibacterial activity of ethanolic and aqueous extracts of *Imperata cylindrica* on the test organisms (*E. coli* and *Staphylococcus* spp). The result shows significant zones of inhibition at higher concentration (200mg/ml and 100mg/ml). The result also shows that the ethanolic extract of *Imperata cylindrica* has the highest zone of inhibition on all organisms, especially *Staphylococcus* spp. at all concentrations of the extract. Table 4 shows the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the ethanolic and aqueous extract of *Imperata cylindrica* on the test organism. The result follows similar trend as that of the antibacterial sensitivity test. That is, the MIC and MBC were found to be at the highest concentration (50% and 25%) only.

Table 1: Results of the phytochemical screening of the ethanolic and aqueous extract of *Imperata cylindrica* roots.

Phytochemicals	Results	
	Ethanolic	Aqueous
Alkaloids	+	+
Reducing sugars	+	+
Cardiac glycosides	+	+
Flavonoids	+	+
Phenol	-	-
Tannins	+	+
Quinones	-	-
Amino acids	-	-
Volatile oils	-	-
Terpenoids	+	+
Saponins	+	+

key + = Present
- = Absent

Table 2: Result of the antibacterial sensitivity test of the ethanolic extract of *Imperata cylindrica* showing zones of inhibition (mm).

Organisms	Concentrations(mg/mL)				
	200	100	50	25	125
E. coli	16.0±1.00	14.0±1.00	10.0±1.00	7.67±0.58	4.0±1.00
Staphylococcus aureus	18.3±1.53	16.0±1.00	10.7±0.58	7.3±1.53	5.5±1.53

Table 3: Result of the antibacterial sensitivity test of the aqueous extracts of *Imperata cylindrica* showing zones of inhibition (mm).

Organisms	Concentrations (mg/mL)				
	200	100	50	25	12.5
E. coli	13.33±0.58	12.0±3.46	8.33±0.58	6.33±0.58	2.5±1.00
Staphylococcus aureus	13.7±1.53	11.7±1.53	10.33±0.58	8.0±1.00	4.0±0.58

Table 4: Result of the MIC and MBC of ethanolic and aqueous extracts of *Imperata cylindrica*

Organisms	MIC (%)		MBC (%)	
	Ethanolic	Aqueous	Ethanolic	Aqueous
E.coli	25	50	50	_
Staphylococcus aureus	25	25	50	50

KEY: MIC=Minimum Inhibitory Concentration, MBC= Minimum Bactericidal Concentration

Herbal medicine has been shown to have genuine utility and about 80% of rural dwellers depend on its efficacy for their primary health care [17]. A medicinal plant contributes an effective source for both traditional and modern-medicines.

The results obtained in this study revealed the phytochemicals present in the aqueous and the ethanolic extracts of *Imperata cylindrica* roots to include; alkaloids, reducing sugars, cardiac glycosides, flavonoids, tannins, terpenoids and saponins as shown in Table 1. This agrees with the study carried out by [18] which opined that the antibacterial activity of *Imperata cylindrica* was found to be dependent on the nature of the solvent used for the extraction and the concentration of the extract.

Ethanolic extract was observed to possess more antibacterial activities compared to the aqueous extract. This may be attributable to the fact that, ethanol extracted more of the bioactive components of the plant compared to the aqueous. Zones of inhibition produced by the ethanolic extract ranged from 4.0 and 5.5 ±1.53 at 12.5 mg/mL to 18.3 ± 1.53 at 200 mg/mL against *Staphylococcus* spp. While the aqueous extract had zones of inhibition ranged from 2.5 ± 1.00 mm at 12.5 mg/mL to 13.3 ± 0.58 mm at 200 mg/mL against *E.coli* and 4.0 ± 0.58 mm at 12. 5mg/mL. It is observed from the result that all the isolates were sensitive to all the extracts.

The bacterial isolates were differently affected by the ethanolic extract and aqueous extracts. This is due to variations in turn affected the degree of phytochemical extracted. The variations observed in the susceptibility testing of Gram positive and negative bacteria could have resulted from their relative composition of cell wall components. Gram positive bacteria have thick peptidoglycan layer while Gram negative bacteria have thick lipopolysaccharide layer. These layers have differential resistance to antibacterial agents.

The minimum Inhibitory Concentration of ethanolic extracts was found to be 25% in *E. coli* and *Staphylococcus aureus* while the aqueous extract was 25% in *Staphylococcus* spp. and 50% *E. coli.* MBC was observed to be at 50% concentration for both ethanolic extract on *E. coli* and *Staphylococcus* spp. which aqueous extract was sensitive only on *Staphylococcus* spp.

Conclusion

This research work has made known that *Imperata cylindrica* has potential bioactive compounds that are responsible for its antibacterial activity. There should be more research carried out on *Imperata cylindrica* roots to enable the purification of the specific bio-potential chemicals and their subsequent processing into chemotherapeutic agents.

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